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Overnight improvements in two REM sleep-sensitive tasks are associated with both REM and NREM sleep changes, sleep spindle features, and awakenings for dream recall

T. Nielsen [a,b,*], C. O'Reilly [a], M. Carr [a,c], G. Dumel [a,d], I. Godin [a,d], E. Solomonova [a,c], J. Lara-Carrasco [a,d], C. Blanchette-Carrière [a,d], T. Paquette [a]

[a] Dream & Nightmare Laboratory, Center for Advanced Research in Sleep Medicine, Hôpital du Sacré-Coeur de Montréal, Canada
[b] Department of Psychiatry, Université de Montréal, Canada
[c] Department of Biomedical Sciences, Université de Montréal, Canada
[d] Department of Psychology, Université de Montréal, Canada

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ABSTRACT

Memory consolidation is associated with sleep physiology but the contribution of specific sleep stages remains controversial. To clarify the contribution of REM sleep, participants were administered two REM sleep-sensitive tasks to determine if associated changes occurred only in REM sleep. Twenty-two participants (7 men) were administered the Corsi Block Tapping and Tower of Hanoi tasks prior to and again after a night of sleep. Task improvers and non-improvers were compared for sleep structure, sleep spindles, and dream recall. Control participants ($N = 15$) completed the tasks twice during the day without intervening sleep. Overnight Corsi Block improvement was associated with more REM sleep whereas Tower of Hanoi improvement was associated with more N2 sleep. Corsi Block improvement correlated positively with %REM sleep and Tower of Hanoi improvement with %N2 sleep. Post-hoc analyses suggest Tower of Hanoi effects—but not Corsi Block effects—are due to trait differences. Sleep spindle density was associated with Tower of Hanoi improvement whereas spindle amplitude correlated with Corsi Block improvement. Number of REM awakenings for dream reporting (but not dream recall per se) was associated with Corsi Block, but not Tower of Hanoi, improvement but was confounded with REM sleep time. This non-replication of one of 2 REM-sensitive task effects challenges both 'dual-process' and 'sequential' or 'sleep organization' models of sleep-dependent learning and points rather to capacity limitations on REM sleep. Experimental awakenings for sampling dream mentation may not perturb sleep-dependent learning effects; they may even enhance them.

1. Introduction

1.1. REM and NREM sleep stages in memory consolidation

Despite strong and growing evidence supporting a role for sleep in the consolidation of new memories, the contribution of specific sleep stages remains controversial (see reviews Diekelmann & Born, 2010; Ellenbogen, Payne, & Stickgold, 2006; Walker & Stickgold, 2010). Many findings support 'dual-process' models which stipulate that REM and NREM stages of sleep facilitate different memory systems, most commonly, hippocampally- vs. non-hippocampally-mediated memories, or non-declarative vs. declarative memories (e.g., Maquet, 2001). To illustrate, in the case of REM sleep, associated improvements have been demonstrated for mirror-tracing (Plihal & Born, 1997), complex logic, word priming, emotional memory (Baran, Pace-Schott, Ericson, & Spencer, 2012; Gujar, McDonald, Nishida, & Walker, 2011; Wagner, Fischer, & Born, 2002; Wagner, Gais, & Born, 2001), and visuospatial working memory (see Smith, 1995 for review), while in the case of NREM sleep, associated improvements have been demonstrated for paired-associate learning (Plihal & Born, 1997), facial recognition (Clemens, Fabo, & Halasz, 2005), face-name, face-scene and face-city associations (Bergmann, Molle, Diedrichs, Born, & Siebner, 2012; Clemens et al., 2005; Ruch et al., 2012), and spatial maze learning (Meier-Koll, Bussmann, Schmidt, & Neuschwander, 1999).

* Corresponding author at: Center for Advanced Research in Sleep Medicine, Hôpital du Sacré-Coeur de Montreal, 5400 Gouin Blvd West, Montreal, Quebec H4J 1C5, Canada. Fax: +1 1 514 338 2531.
E-mail address: tore.nielsen@umontreal.ca (T. Nielsen).

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In contrast, many results support alternative models according to which both REM and NREM sleep are required for memory consolidation. These alternatives include 'sequential' or '2-stage' models that require a succession of REM and NREM stages (Buzsaki, 1989; Fogel, Smith, & Beninger, 2009; Giuditta, Mandile, Montagnese, Piscopo, & Vescia, 2003), and 'sleep organization' models requiring an intact organization of NREM-REM cycles through the night of sleep (Ficca & Salzarulo, 2004). Some supportive findings include the facts that in humans visual discrimination learning is associated with changes in both NREM and REM sleep on the same night (Stickgold, Whidbee, Schirmer, Patel, & Hobson, 2000), and that in rats avoidance learning is followed by increases in both REM sleep theta power and NREM sleep spindle density (Fogel et al., 2009). Such alternative models also account more parsimoniously for results not easily explained by dual-process approaches, e.g., that declarative memory can at times be associated with REM (rather than NREM) sleep (Tilley & Empson, 1978) and that non-declarative memory can at times be associated with NREM (rather than REM) sleep (Doyon et al., 2009; Morin et al., 2008; Smith & MacNeill, 1994; Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002).

Clarifying the role of sleep stages in memory consolidation is further complicated by the multiplicity of macro- and micro-structural sleep measures and their differential associations with different learning tasks within and between experiments. Memory improvements have been linked not only to changes in the relative proportions of REM sleep (Baran et al., 2012; Gujar et al., 2011), Stage 2 (N2) sleep (van der Helm, Gujar, Nishida, & Walker, 2011), and Stages 3 and 4 (N3) sleep (Lau, Tucker, & Fishbein, 2010), but also to particular sleep features such as EEG spindles (Barakat et al., 2011; Fogel & Smith, 2011), theta waves (Popa, Duvarci, Popescu, Lena, & Pare, 2010), alpha and beta EEG power (Yordanova, Kolev, Wagner, Born, & Verleger, 2012), slow (<1 Hz) EEG oscillations (Dickson, 2010; Marshall, Helgadottir, Molle, & Born, 2006), and rapid eye movement density (Smith, Nixon, & Nader, 2004). Even different attributes of each measure have found to be differentially related to learning; to name a few: REM density vs. duration (Fogel et al., 2009), early vs. late night REM or NREM sleep (Stickgold et al., 2000), spindle density vs. duration (Bergmann et al., 2012), or variations in cortical topography of EEG changes (Murphy et al., 2011). Thus, it is not unusual to find that two different sleep measures are associated with cross-night improvements on two different learning tasks. To illustrate, one study reported that among the same participants, overnight improvements on a face recognition task were associated with NREM sleep time, whereas improvements on a face-name associates task were linked to an increase in localized stage N2 sleep spindles (Clemens et al., 2005). More recent models of sleep-sensitive learning have attempted to deal with this complexity (Stickgold & Walker, 2013), but more research is clearly needed.

Thus, despite burgeoning evidence supporting a role for sleep in memory consolidation, the contribution of specific sleep stages, or combinations of sleep stages, to memory remains contentious. Different models have been proposed to account for the variety of findings but none is predominant. A promising strategy for examining the sleep stage/memory type question is the use of several tasks with known sleep-dependent effects in conjunction with the assessment of multiple sleep variables concurrently. The present protocol employs such a strategy in administering two validated tasks for which overnight improvement is dependent upon REM sleep (Smith, 1995) but which index different cognitive and brain systems: the Corsi Block Tapping Task (CBT) (Milner, 1971) and the Tower of Hanoi (ToH) (Cohen, Eichenbaum, Deacedo, & Corkin, 1985). These tasks are both non-verbal in nature and both draw upon working memory capacity. However, the CBT is a visuospatial working memory task sensitive to hippocampal functioning (Toepper et al., 2010) while the ToH is a problem-solving, executive function task sensitive to frontal lobe functioning (Milner, 1971; Welsh, Satterlee-Cartmell, & Stine, 1999). Dual-process models would lead to the expectation that both of these tasks will demonstrate an association with overnight REM but not NREM sleep. Alternative 2-stage or sequential models might predict that the two tasks will be associated with both REM and NREM sleep measures.

1.2. Dream mentation sampling as a possible experimental artifact

Some research has demonstrated memory consolidation to be followed by changes in dream mentation that is sampled from either REM (De Koninck, Christ, Rinfret, & Proulx, 1988; Fiss, Kremer, & Lichtman, 1977; Pantoja et al., 2009) or NREM (Wamsley, Tucker, Payne, Benavides, & Stickgold, 2010) sleep. Compared with the sleep studies described above, dream mentation research is less common (see reviews in Smith, 2010; Wamsley & Stickgold, 2011) in part because the awakening of participants for mentation sampling disturbs the associated sleep physiology and may thus perturb sleep-related memory benefits. However, it is also possible that awakenings from sleep will enhance memory; studies with rats have found that sequences of REM and NREM sleep that include transitions to wakefulness are associated with the fast learning of avoidance reactions (Piscopo et al., 2001). Given the paucity of information about the effects of night awakenings and of recalling dream mentation on memory in humans, our protocol was designed to assess whether these factors were associated with disruption or enhancement of REM sleep-dependent effects on performance for two tasks.

2. Methods

2.1. Participants

Thirty-seven healthy volunteers were recruited by advertisements in newspapers and by word of mouth. They reported themselves to be free of sleep, psychiatric, and physical illnesses, to have normal sleep schedules, and to be free from medications. They were reminded several days prior to participating to abstain from alcohol for at least 24 h, and from caffeine for at least 6 h, prior to arriving at the laboratory. The sleep study sample ($N = 22$) comprised two cohorts. Sixteen participants (4 men, 12 women) spent 2 nights each in the sleep laboratory with cognitive testing on night 1. Six additional participants (3 men, 3 women) spent 1 night each in the laboratory with the same cognitive testing on that night. The two cohorts did not differ in age or on any sleep or cognitive test measures and were combined to form a sample (mean age: 25.0 ± 5.0), of whom 15 were women (mean age: 25.1 ± 5.5) and 7 were men (mean age: 24.6 ± 4.2). The waking state control sample ($N = 15$) consisted of 11 women and 4 men with a mean age of 24.9 ± 5.6 yrs. The sleep and wake groups did not differ in age. All participants gave written informed consent; the study was approved by the hospital ethics review board.

2.2. Procedures

2.2.1. Cognitive testing

Participants in the sleep sample arrived at the sleep laboratory at least 2 h prior to their normal bed times. A polysomnographic (PSG) recording montage was applied and 2 cognitive tasks were administered 30 min before lights out (T1-S). These were the Tower of Hanoi (ToH) task (Cohen et al., 1985) followed by the Corsi Block Tapping (CBT) task (Milner, 1971). For the ToH, participants were told that a pyramid of 5 disks should be moved from

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the 1st peg to the 3rd peg of a 3-peg array according to the following rules: (a) a disk must be moved from one peg to another, (b) only one disk may be moved at a time, and (c) a larger disk may never be placed on top of a smaller disk. An assistant noted each participant's time and number of moves to completion. The optimal solution for the ToH ($2^n - 1$, where n = #disks) was 31 moves for this configuration. The tasks were readministered in the same order 30 min after final awakening in the morning (T2-S). Due to technical problems, 3 participants had missing observations for the ToH evening or morning scores.

For the CBT, the experimenter initiated the first level by tapping out a three-block sequence with the index finger, the order of which participants were asked to repeat. Two trials were given and then the sequence was lengthened by one block. The test was discontinued when both levels of a sequence were failed. A score of 1 was assigned for each correctly recalled sequence. For the CBT forward subtest, participants were given credit for both untested 2-block sequences. For the CBT backward subtest, participants were started with a 2-block sequence and asked to repeat it in reverse order. Sequence length was again lengthened after 2 successful trials.

Following testing, participants prepared for bed and the lights were extinguished. Awakenings to sample dream mentation were conducted throughout the night according to a fixed protocol (see later).

Waking state control participants were given the two tasks in the same order at two times of the day that were separated by approximately the same time (mean duration: 8:13 ± 0:29 h) that separated the two testing administrations for the sleep groups, i.e., around 10:26 ± 2:16 AM (mean ± SD) in the morning (T1) and around 6:39 ± 2:19 PM in the afternoon (T2). Participants were instructed not to nap between administrations.

Performance change scores were calculated by subtracting T1 from T2 scores for the following 5 variables: ToH number of moves to completion (ToH_{moves}), ToH time to completion (ToH_{time}), CBT total, CBT forwards, CBT backwards; ToH_{time} and CBT total were selected as primary endpoints for these analyses.

2.2.2. PSG recording and sleep staging

PSG recordings were accomplished with a montage of 19 standard 10–20 EEG channels (Fp1, Fp2, F3, F4, Fz, F7, F8, C3, C4, Cz, P3, P4, Pz, T3, T4, T5, T6, O1, O2) referenced to A1 + A2 with a 10-kΩ resistance for cohort 1 and 6 standard channels (F3, F4, C3, C4, O1, O2) referenced to A1 for cohort 2. Recordings also included EOG, submental EMG, and EKG leads. Biosignals were recorded through the night using a Grass 12 Neurodata Acquisition System (−6 dB filters with cut offs at 0.30 [time constant: 0.4 s] and 100 Hz) and archived under the control of Rhythm 9.1 (cohort 1) or Harmonie 5.4 (cohort 2) software (Stellate systems, Montreal, Canada). PSG tracings were scored by an experienced PSG technician using AASM (2007) criteria (Silber et al., 2007). Standard sleep variables (stage REM min, %REM, Stage N2 min, %N2, etc.) were calculated by in-house software.

2.2.3. Sleep spindle analysis

In-house software (Martin et al., 2013) was used to detect, count and calculate the amplitude of all sleep spindles occurring in stages N2 and N3 sleep on 3 left (F3, F4, C3) and 3 right (C4, O1, O2) channels. Spindles were detected automatically using an 11–15 Hz band pass filter with a linear phase Finite Impulse Response filter (order 511, rectangular window, −3 dB at filter limits). Forward and reverse filtering was performed to eliminate phase distortion and to double the filter order. The root mean square (RMS) of the filtered signal was calculated with a 0.10-s time window. A spindle was identified when 5–40 consecutive RMS time points – i.e., a 0.5–4.0 s time duration – exceeded a threshold fixed at the 95th percentile of the RMS amplitude for the sleep cycle and stage that incorporated the spindle. Spindle counts for each channel were subsequently divided by total number of minutes elapsed in the corresponding sleep stage to produce density scores. To assess relationships between spindles and sleep-dependent learning, Spearman correlations were calculated between spindle density and amplitude scores and T2-T1 performance change scores for ToH_{moves}, ToH_{time}, and CBT total, CBT forward and CBT backward scores using a $p < .05$ significance threshold. To control for possible trait differences between groups, follow-up partial correlations that covaried baseline (T1) performance were also conducted.

2.2.4. Awakenings for dream recall

Participants were awakened from sleep for dream collection when a trained judge, unaware of the hypotheses of the study, determined that one of 4 sleep stage conditions had been met. The judge targeted one each of Stage REM and Stage N2 sleep episodes from early in the night (first 3.5 h) and one each from late in the night (second 3.5 h). Pairs of REM and N2 awakenings were matched as closely as possible for duration since stage onset and time since lights out over nights (cohort 1) or over yoked participants (cohort 2) and counterbalanced for order. Minimum duration of time in stage was 5 min for early REM periods and 10 min for all others. The judge used standard Rechtschaffen and Kales (1968) sleep staging criteria to determine in real-time if either a REM or N2 episode of sufficient duration had occurred. These decisions were verified off-line by a second trained judge.

Each participants was awakened using an 80 dB tone and instructed by intercom to recall everything that was going on in his/her mind just before the awakening beginning with the last 30 s of the dream and followed by whatever preceded that. After a 30-s delay, dim lights were turned on and a verbal report was elicited. If a participant stopped talking, he or she was asked if there was anything else they would like to add. The participant was then asked to verbally respond to several questions about the vividness and emotional intensity of the mentation using Likert-type scales. These ratings are not reported here. Verbal reports were recorded, transcribed and assessed by a judge who was blind to the conditions and hypotheses for whether or not the report contained some pseudo sensory content or thoughts. If so, the report was scored 1; if not, it was scored 0. For the purposes of this paper 'dream mentation' is used as a term for any content scored as 1 from either REM or N2 sleep. For each participant, the following variables were calculated: total #awakenings, %awakenings producing dream mentation, #awakenings early, #awakenings late, #REM awakenings, %REM awakenings producing dream mentation, #N2 awakenings, %N2 awakenings producing dream mentation.

2.3. Statistical analyses

Analyses were conducted using Statistica 6.0 and SPSS 19.0 and are described individually in the Results section. Non-parametric tests were applied when distributions were non-Gaussian.

3. Results

3.1. ToH and CBT performance changes

For the sleep groups, there was a decrease in ToH_{moves} from evening (80.6 ± 42.2) to morning (55.8 ± 28.8; Wilcoxon Matched Pairs (WMP) test = 41.00, $p = .030$) as well as a shortening of ToH_{time} from evening (347.2 ± 200.7 s) to morning (183.1 ± 159.9; WMP test = 16.0, $p = .001$). As shown in Fig. 1, upper panel, a

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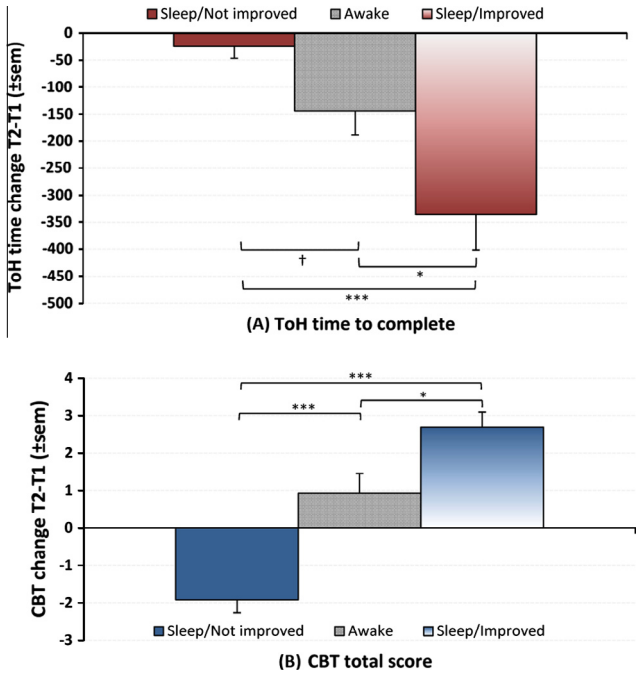

Fig. 1. Mean change (T2-T1) scores (±sem) for Tower of Hanoi (ToH) time to complete (upper panel) and Corsi Block Tapping (CBT) total score (lower panel) for Improved and Not improved sleep groups and Awake control group. $^{***}p < .001$; $^{*}p < .05$; $^{\dagger}p = .096$.

median split on the T2-T1 change scores for ToH_{time} showed one group ($n = 10$) with improved performance (M_{change}: −335.8 ± 198.8 s) and a second group ($n = 9$) with reduced or no change in performance (M_{change}: −24.7 ± 62.1; standardized Mann–Whitney $U(Z) = -3.67$, $p < .001$). The Awake group showed a moderate improvement (M_{change}: −144.1 ± 172.0) which differed both from Sleep/Improved (MW $Z = 2.18$, $p = .030$) and, marginally, Sleep/Not improved groups (MW $Z = -1.66$, $p = .096$). The same grouping also showed improved performance on ToH_{moves} (M_{change}: −56.9 ± 45.8 vs. 1.5 ± 12.1; MW $Z = -3.08$, $p < .002$) with a moderate improvement for the Awake group (M_{change}: −15.2 ± 30.0) that differed significantly from the Sleep/Improved group (MW $Z = 2.24$, $p = .025$) and marginally from the Sleep/Not improved group (MW $Z = -1.61$, $p = .108$). ToH_{time} was used as the endpoint for subsequent analyses.

In contrast, CBT total score for the sleep sample failed to improve from the evening (M: 17.45 ± 3.28) to the morning (M: 17.64 ± 3.54) testing sessions ($t_{21} = -0.182$, $p = .749$). This was also true for the CBT forward (8.91 ± 2.09 vs. 9.18 ± 2.20, $t_{21} = -0.273$, $p = .444$) and backward (8.55 ± 1.65 vs. 8.45 ± 1.92, $t_{21} = 0.302$, $p = .765$) subtests. As shown in Fig. 1, lower panel, a median split on the T2-T1 change for CBT total scores produced one group of sleep participants ($n = 10$) with improved performance (M_{change}: +2.70 ± 1.25) and a second group ($n = 12$) with reduced or no change in performance (M_{change}: −1.92 ± 1.16; MW $Z = 3.956$, $p < .00001$). For CBT total score, the Awake group (M_{change}: 0.93 ± 2.05; $n = 15$) differed from both the Sleep/not improved (MW $Z = 3.367$, $p = .0008$) and Sleep/improved (MW $Z = -2.191$, $p = .028$) groups. The Awake group also differed from the two Sleep groups on CBT forwards (MW $Z = 2.171$, $p = .030$ for the Sleep/not improved group; $F_{34} = -1.858$, $p = .063$ for the Sleep/improved group). However, for CBT backwards, the Awake group differed from the Sleep/not improved group (MW $Z = 3.074$, $p = .002$) but not the Sleep/improved group (MW $Z = -0.804$, $p = .421$). CBT total was used as the endpoint for subsequent analyses.

Improvement levels on the 2 tasks were independent of one another. Improved and not improved ToH groups did not differ on any CBT improvement measure (Mann–Whitney, all $p > .916$). Nor did improved and not improved CBT groups differ on ToH_{time} (Mann–Whitney, $p = .624$) or ToH_{moves} ($p = .496$). Further, there were no significant correlations between change on the CBT task (total) and change on the ToH_{time} ($r = -.077$, $p = .754$) or ToH_{moves} ($r = -.055$, $p = .818$) measures.

3.2. Performance changes associated with sleep variables

3.2.1. ToH task

PSG measures for participants who did and did not improve on ToH_{time} are shown in Table 1 and Fig. 2 panel A. REM sleep measures did not distinguish between groups. However, N2 sleep minutes and %N2 sleep were higher for improved than for not improved participants, while %N3 sleep were lower. The N2 sleep differences (min and %) occurred exclusively in the second half of the sleep period, whereas the N3 sleep difference (%) was primarily for the first half, suggesting that late-night N2 sleep increased to the detriment of early-night N3 sleep following ToH task exposure.

Further supporting this notion, %N2 and %N3 sleep were negatively correlated (Pearson $r_{22} = -.647$, $p = .001$), especially %N2 for the 2nd half of the sleep period with %N3 for the 1st half ($r_{22} = -.527$, $p = .012$) as opposed to %N2 with %N3 for the 2nd half ($r_{22} = .179$, $p = .424$).

N2, but not REM, sleep minutes correlated with improved performance (lower time) on ToH_{time} ($r_{19} = -.408$, $p = .083$); a non-significant trend in the same direction was observed for ToH_{moves} ($r_{20} = -.309$, $p = .186$). As shown in Fig. 3 (panel C), similar negative correlations were obtained for %N2 sleep, i.e., ToH_{time}: $r_{19} = -.468$, $p = .043$; ToH_{moves}: $r_{20} = -.237$, $p = .314$.

To control for possible trait differences in ToH performance that might explain the observed relationships with sleep variables, group comparisons on PSG variables were redone with presleep ToH time scores—a presumed index of trait differences—as a covariate using an ANCOVA design. The main effect for %N2 sleep reported in Table 1 was reduced to a trend ($F_{1,17} = 2.975$, $p = .103$) although the complementary main effect for %N3 sleep was not ($F_{1,17} = 5.351$, $p = .033$). Also, with evening ToH time performance partialed out, %N2 sleep no longer correlated with ToH_{time} ($r_{16} = -.109$, $p = .667$) or ToH_{moves} ($r_{16} = .039$, $p = .879$) and there were no significant correlations for %N3 sleep or for the 1st and 2nd halves of the night (all $p > .353$).

3.2.2. CBT task

Standard PSG measures for participants who did and did not improve on the CBT are shown in Table 2 and Fig. 2 panel B. Performance differences were clearly associated with REM sleep minutes and %REM sleep (Fig. 2 panel B), marginally so with total sleep time, and not at all with any NREM sleep measures. The pattern of REM sleep differences was seen for both the 1st and 2nd halves of the night (Table 2).

Spearman correlations revealed positive associations between REM sleep minutes and CBT total ($r_{22} = .662$, $p = .001$), CBT forwards ($r_{22} = .554$, $p = .007$), and CBT backwards ($r_{22} = .584$, $p = .004$) scores. As shown in Fig. 3, panels A and B, these correlations were also evident, albeit slightly reduced, for the %REM sleep measure. Correlations with N2 min and % were all negative (Fig. 3, panel A).

To assess whether the CBT group effect reflected a trait difference common to both memory improvement and sleep architecture, the CBT groups were compared on PSG measures using evening CBT total scores as covariates. This possibility was not supported in that the group differences reported in Table 2 remained significant for %REM ($F_{1,19} = 5.41$, $p = .031$) and REM minutes

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Table 1
Sleep stage measures for participants who did and did not improve on time to complete the Tower of Hanoi Task (ToH_{time}). Both minutes and % of N2 sleep were higher and minutes and % of N3 sleep were lower for the improved group. REM sleep measures did not differentiate groups. N2 sleep differences occurred in the 2nd half of the night whereas N3 sleep differences occurred in the 1st half. Independent groups *t*-test comparisons (all Levene's tests for variance equality NS).

		ToH_{time} improved		ToH_{time} not improved		
		Mean	SD	Mean	SD	*p*
Total sleep time	min	379.4	72.1	359.9	57.8	.501
Stage N1	min	43.3	25.4	36.8	15.1	.482
	%	11.7	6.9	10.3	4.0	.577
Stage N2	min	208.4	45.2	167.4	33.5	.028*
	%	55.1	6.4	46.9	8.7	.026*
– 1st half of night	min	98.5	29.3	83.0	21.2	.181
	%	25.4	4.9	23.6	6.9	.497
– 2nd half of night	min	110.1	16.7	84.4	21.1	.006**
	%	29.7	5.2	23.4	4.3	.006**
Stage N3	min	60.6	25.7	83.2	30.9	.086t
	%	16.1	5.8	23.0	7.3	.027*
– 1st half of night	min	49.4	27.8	67.0	21.8	.121
	%	12.6	6.3	18.6	5.3	.026*
– 2nd half of night	min	11.3	8.2	16.2	13.2	.320
	%	3.5	3.7	4.4	3.6	.587
Stage REM[a]	min	67.1	29.4	72.5	29.7	.678
	%	17.1	5.6	19.7	6.2	.331

[a] No group differences for REM sleep for the 1st and 2nd halves of the night were observed.
* $p < .05$.
** $p < .01$.

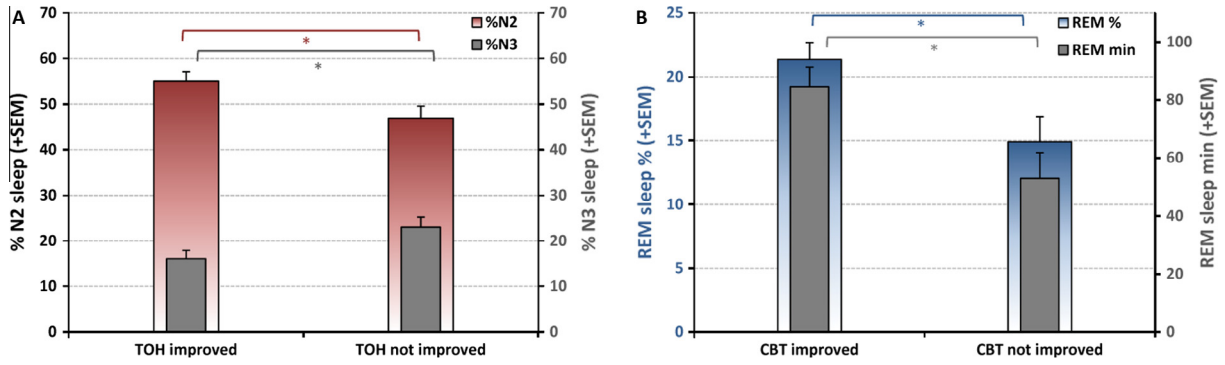

Fig. 2. A. Differences in %N2 and %N3 sleep for participants who did not show evening-to-morning improvement on the Tower of Hanoi (ToH) time to completion . %N2 was elevated and %N3 reduced in improved participants. B. REM sleep amounts (minutes and %) for participants who did not improve on the Corsi Block Tapping (CBT) Task. CBT improved participants had more REM sleep on both measures. * $p < .05$.

($F_{1,19} = 5.80$, $p = .026$). Further, with evening CBT total score partialed out, %REM sleep continued to show large positive correlations with improvements in CBT total ($r_{19} = .566$, $p = .007$), CBT forwards ($r_{19} = .511$, $p = .018$), and CBT backwards ($r_{19} = .452$, $p = .040$) scores.

3.3. Performance changes associated with N2 and N3 sleep spindle density and amplitude

Positive Spearman correlations between spindle density scores and T2-T1 change scores for ToH_{time} and ToH_{moves} indicated that more dense spindles were associated with better overnight improvement (Fig. 4, upper panels). Thirteen of the 24 coefficients were positive at $p < .05$ while one was marginally so ($p = .067$). For ToH_{moves}, relationships were restricted to N3, where all but one channel (C4) were positively correlated at $p < .05$. For ToH_{time}, frontal (F3, F4) and left central (C3) spindle densities were correlated with faster times for both N2 and N3 stages. Additionally, occipital (O1, O2) densities were correlated with faster times for N3 only. Partial correlations covarying baseline (T1) scores indicated that these relationships did not entirely reflect pre-existing group differences in baseline performance. As indicated by the parenthesized asterisks in Fig. 4, with this control 9 of the 13 identified correlations remained significant at $p < .05$. No significant correlations with ToH scores were observed for spindle median amplitude (all $p > .147$).

Spearman correlations between spindle median amplitude and change scores for CBT total and CBT backwards are shown in the lower panels of Fig. 4. Although fewer coefficients than for the ToH exceeded the $p < .05$ threshold, i.e., 8 of 24 coefficients, a consistent pattern of negative relationships with CBT backwards scores (right panel) indicated that CBT improvement was associated with lower amplitude spindles in frontal (F3, F4) and central (C3, C4) channels during both N2 and N3 sleep—although the correlation for C3 during N2 was only marginal ($r_{22} = -.340$, $p = .096$). For CBT total score improvement, a similar pattern of negative correlations for frontal and central channels was seen (left panel), but only the correlation for C4 during N3 ($r_{22} = -.435$, $p = .043$) surpassed the $p < .05$ threshold. No substantive correlations were observed for changes in CBT forwards scores (all $p > .350$). The majority of correlations observed were not diminished by partialing out performance scores at T1.

No correlations with spindle density were observed in either N2 or N3 sleep or for any channel for CBT total scores (all $p > .16$), CBT forwards scores (all $p > .27$), or CBT backwards scores (all $p > .19$).

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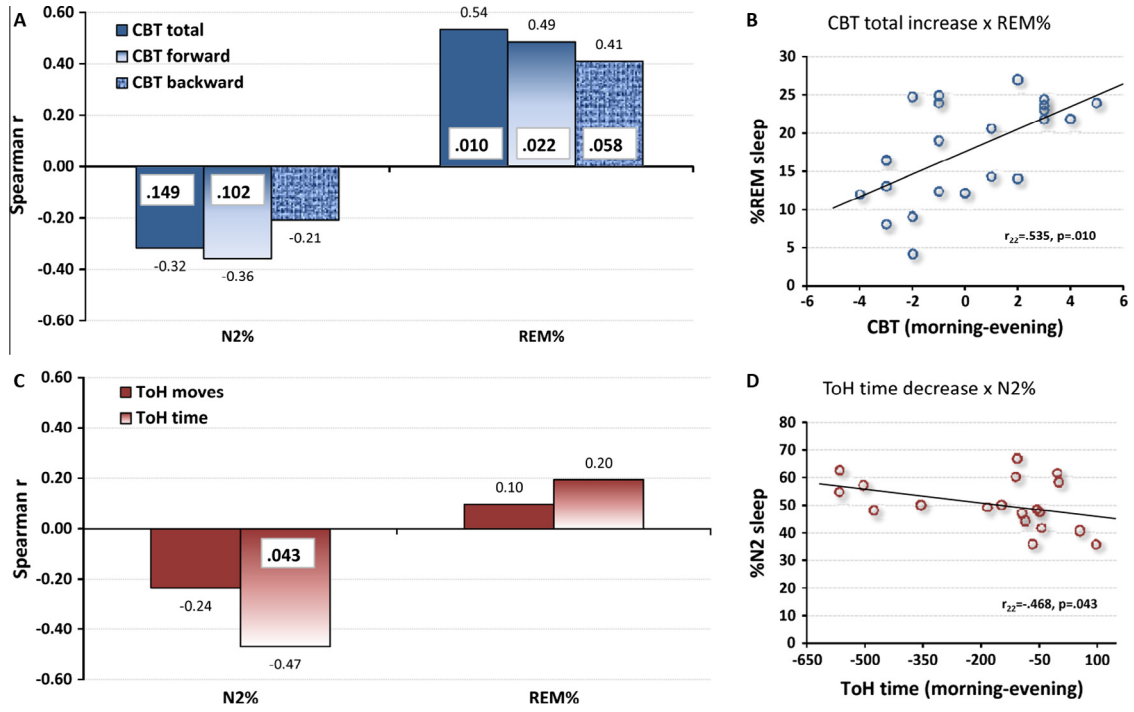

Fig. 3. Spearman correlations between overnight changes in task performance and sleep stage percentages. A and B: %REM sleep (%REM) correlated with improvements (longer sequences) on Corsi Block Tapping (CBT) total score, CBT forwards, and CBT backwards. C and D: %N2 sleep (%N2) correlated primarily with improvements on Tower of Hanoi (ToH) time to completion (reduced time). *r*-values are shown above and below columns; *p*-values <.150 are shown in column insets.

Table 2
Sleep stage measures for participants who did and did not improve on the Corsi Block Tapping Task (CBT). Both minutes and % of REM sleep were higher for the CBT improved group. NREM sleep measures did not differentiate groups. REM sleep differences occurred in both the 1st and 2nd halves of the night. Independent groups *t*-test comparisons (all Levene's tests for variance equality NS).

		CBT improved		CBT not improved		
		Mean	SD	Mean	SD	*P*
Total sleep time	min	393.5	50.5	340.7	69.7	.060§
Stage N1	min	41.5	14.2	37.3	24.5	.634
	%	10.7	3.6	11.0	6.6	.887
Stage N2[a]	min	190.9	33.1	179.6	52.3	.562
	%	48.5	6.3	52.8	9.6	.242
Stage N3[a]	min	76.6	25.7	70.8	33.7	.660
	%	19.4	5.7	21.2	9.6	.603
Stage REM	min	84.5	21.9	53.0	30.6	.013*
	%	21.4	4.2	14.9	6.9	.018*
1st half	min	23.2	12.7	12.3	10.0	.036*
	%	5.9	3.2	3.3	2.6	.047*
2nd half	min	61.3	19.0	40.7	22.6	.033*
	%	15.4	3.9	11.6	5.2	.072§

[a] No group differences for 1st and 2nd halves of the night were observed for N2 and N3 sleep.
* $p < .05$.
§ $p < .10$.

3.4. Performance changes associated with REM and N2 awakenings for dream mentation collection

There were 79 awakenings for dream sampling from REM and N2 sleep; the average rate of mentation recall for all awakenings was 66.8 ± 32.3%. Only 1 participant (5%) had no recall on any awakening. 36 awakenings were from REM sleep (recall rate: 83.3 ± 29.2%); 43 awakenings were from N2 sleep (recall rate: 65.6 ± 40.1%)—a significant difference (paired $t_{15} = 2.15$, $p = .048$). 27 awakenings were from early in the night (recall rate: 57.9 ± 44.9%); 52 awakenings were from late in the night (recall rate: 71.5 ± 37.2%)—a non-significant difference ($t_{18} = -1.30$, $p = .211$).

3.4.1. ToH task and awakenings

A 2 × 2 × 2 ANOVA with stage (REM, N2) and time of night (early, late) as repeated measures, ToH group (improved, not improved) as independent measure and #awakenings for mentation recall as dependent measure revealed a time of night main effect ($F_{1,19} = 32.36$, $p = .00003$; eta$^2 = .630$) but no main effect or interactions for ToH group (all $p > .364$). Mann–Whitney *U* Tests revealed that there were no differences between ToH improved and not improved groups on any dream recall measure (all $p > .11$).

3.4.2. CBT task and awakenings

A similar ANOVA for the CBT task revealed 2 main effects and no interactions. A main effect for time of night ($F_{1,20} = 33.99$, $p = .00004$; eta$^2 = .630$) indicated more awakenings late (*M*: 2.36 ± 0.66) than early (*M*: 1.23 ± 0.75) in the night. However, an additional main effect for CBT group ($F_{1,20} = 7.35$, $p = .013$; eta$^2 = .269$), accounting for 26.9% of the explained variance, revealed more awakenings for the improved (4.20 ± 1.03) than the not improved (3.08 ± 0.90) group. The latter effect was found for REM sleep awakenings ($F_{1,20} = 4.13$, $p = .056$; eta$^2 = .171$) but not for N2 awakenings ($F_{1,20} = 0.26$, $p = .616$; eta$^2 = .013$). To determine if the CBT group effect was confounded with time in REM sleep—i.e., the improved group having had more REM sleep also had more opportunities for experimental awakenings from REM sleep—REM minutes was controlled as a covariate in the previous analysis. This resulted in a substantial diminishment of the CBT group main effect ($F_{1,19} = 2.27$, $p = .148$; eta$^2 = .107$), i.e., a drop in the explained variance from 26.9% to 10.7%.

Conversely, to determine if the robust difference between CBT improved and not improved groups on REM minutes reported in Table 1 was affected by this confound by #awakenings, one-way ANCOVAs with CBT group (improved, not improved) as

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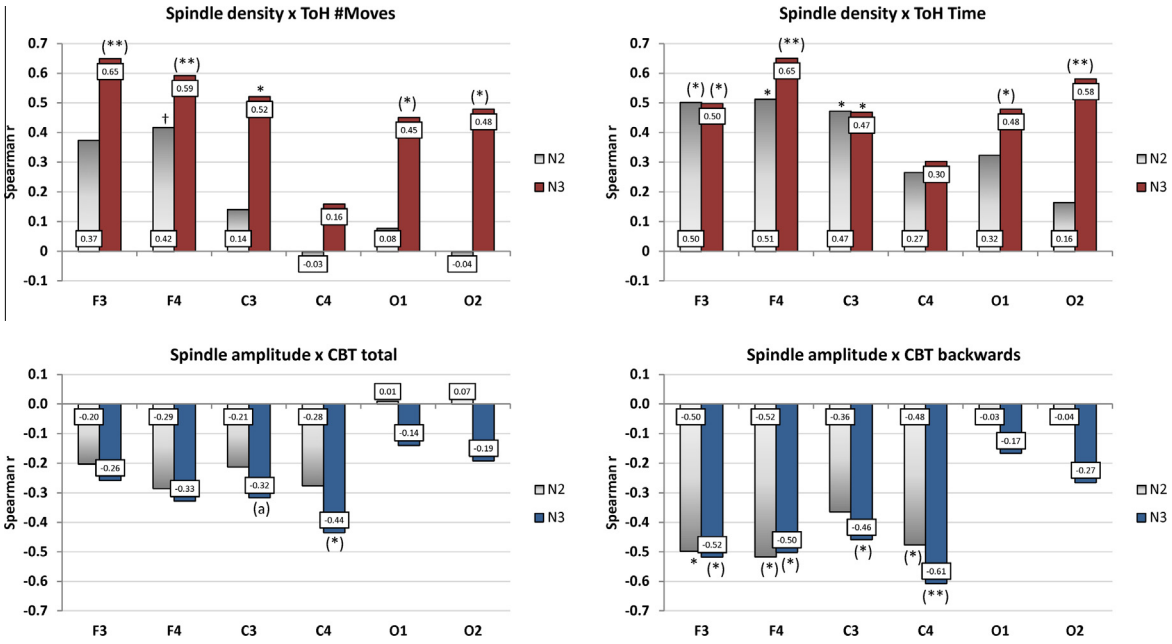

Fig. 4. Spearman correlations between N2 and N3 spindle characteristics and overnight performance change scores. Upper panels: Tower of Hanoi (ToH) #Moves and Time to completion correlations with spindle density. Higher densities for most channels during N3 were associated with decreased ToH #Moves (left); higher densities in frontal channels during both N2 and N3 and in occipital channels during N3 were associated with ToH improved speed (right). Lower panels: Lower median amplitudes in frontal and central channels during N2 and N3 were associated with improved CBT backwards sequences (right); only C4 during N3 sleep was associated with CBT total score (left). Correlations were largely maintained when baseline performance was removed as a covariate (asterisks in parentheses). Correlation coefficients are shown in insets. $^{**}p < .01$; $^{*}p < .05$; $^{\dagger}p = .067$; (a) coefficient significant at $p = .043$ only after partial correlation.

independent variable, REM minutes as dependent variable, and #REM awakenings as covariate were conducted. This reduced the CBT group effect to a trend ($F_{1,19} = 3.73$, $p = .068$). The same was true for the %REM sleep measure ($F_{1,19} = 3.02$, $p = .098$). When assessed by halves of the sleep episode, the covariate eliminated the group effect for the 1st half ($F_{1,19} = 1.26$, $p = .277$) but not for the 2nd half ($F_{1,19} = 3.55$, $p = .075$). Further, #REM awakenings was positively but only moderately correlated with both REM minutes ($r_{22} = .491$, $p = .020$) and %REM sleep ($r_{22} = .470$, $p = .027$). In sum, the #awakenings confound does not completely explain the observed association between REM time and CBT improvement.

Due to an insufficient number of early night awakenings, a similar ANOVA design was not feasible for assessing whether the % of awakenings with recalled dream mentation was associated with CBT improvement. However, Mann–Whitney *U* Tests revealed that there were no differences between CBT improved and not improved groups on any dream recall measure, including %REM dream recall, %N2 dream recall, %early dream recall, %late dream recall or any of the 4 cells of the stage × time of night design (all $p > .37$).

In sum, although improved performance on the CBT was associated with significantly more experimental awakenings, this difference was confounded with the concurrent increase in REM sleep time, i.e., to more opportunities for dream mentation sampling in the improved group. But the confound could not explain away the initial association between REM time and CBT improvement. Further, the extent to which participants were successful in recalling dreams during the awakenings was not associated with CBT or ToH improvement.

4. Discussion

The present findings demonstrate that two REM sleep-sensitive tasks administered in a single testing session did not both prove to be associated with REM sleep changes as might be expected from dual-process models of memory consolidation. They replicate one previous finding that REM sleep is associated with the Corsi Block Tapping task, but fail to replicate a similar finding associating REM sleep with the Tower of Hanoi task (Smith, 1995; Smith et al., 2004). Rather, the Tower of Hanoi task was found to be associated with elevated N2 and reduced N3 sleep and an increase in the density of sleep spindles in these two sleep stages. The present findings also do not support predictions of sequential models of sleep consolidation in that improvements on the two tasks were associated with time in either REM or NREM sleep. One exception to this is that improvement on the Corsi Block Tapping task was also associated with lower spindle amplitudes in NREM sleep. Thus, the CBT may depend upon elements of both REM and NREM sleep, i.e., elevated REM time and lower NREM spindle amplitude.

This incomplete replication of previous findings for REM sleep-dependent learning effects challenges the notion that specific types of memory are necessarily and consistently associated with the same sleep stage. Rather, it suggests that sleep stage/learning associations are mediated by other factors that still require clarification. This suggestion has been mentioned by others (Ackermann & Rasch, 2014); as findings accumulate, it is becoming increasingly evident that a simple one-to-one correspondence between learning tasks and sleep stages is too simplistic to explain all findings. Three factors that might explain the present findings include: (1) limited processing capacity of REM sleep; (2) task competition; (3) participant differences in task aptitude.

(1) *Limited processing capacity of REM sleep*: The unexpected association of the REM-sensitive ToH task with N2 and (inversely) N3 sleep may be due to inherent limitations on the amount of information that REM sleep can process in one night. Both CBT and ToH tasks draw upon working memory capacity, which is correlated with sleep-related memory improvements (Fenn & Hambrick, 2012), and if the combined administration of these tasks placed excessively high demands on working memory capacity in REM sleep, then consolidation of one of

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the tasks—the ToH in this case—may have been disrupted or reprioritized. In such a scenario, the ToH may have been relegated to processing by NREM sleep as an automatic strategy change or as a simple 'default' mechanism. This possibility is consistent with evidence that features of REM sleep appear to vary as a function of learning conditions. For example, the temporal REM sleep 'windows' during which new learning is optimally processed can be delayed by as much as 53–56 h if task training is distributed over several trials or days (Smith, 2003). Or, if levels of task training are low, improvement on an habitually REM sleep-dependent task (brightness discrimination avoidance) can be reversed in rodents, such that improvement occurs only after REM sleep deprivation (Smith & Gisquet-Verrier, 1996). A related possibility is that the order in which the tasks were originally administered determined their processing priority. Such a mechanism is demonstrated in a study in which memory for the 2nd, but not the 1st, of two similar word-lists was associated with later increases in N2 sleep—even though the order of the word-lists was counterbalanced (van der Helm et al., 2011). In our study, however, the first administered task (ToH) was associated with increases in N2 sleep.

Yet another possibility is that when competition occurs between tasks and one of the two is relegated to processing by NREM sleep, that the latter is dealt with by REM sleep only on a subsequent night. Such a possibility would be consistent with 2-stage memory models that stipulate the involvement of both REM and NREM sleep but at different times (Fogel et al., 2009). Such 'stage-switching' of consolidation dependency might explain why we found no association between ToH improvement and REM sleep when retest was after only one night of sleep whereas Smith (1995) and Smith et al. (2004) did find such an association when retest occurred after a 1-week delay. Participants in the Smith et al. studies presumably had 7 nights on which the ToH task could have been processed by REM sleep whereas our participants had only a single opportunity.

(2) *Task competition*: The REM-sensitive tasks administered in the present study may not both have been associated with REM sleep because they implicate different neurocognitive systems. The CBT is generally considered to be a visuospatial working memory task that is sensitive especially to right hippocampal functioning (Toepper et al., 2010) whereas the ToH is an executive function task sensitive to frontal lobe functioning (Milner, 1971; Welsh et al., 1999). The CBT is distinguished by its reliance on spatial, rather than simply serial, memory. Thus, overnight improvements on the WAIS digit span task—a similar but purely rote serial memory task that is independent of the hippocampus (Cave & Squire, 1992)—is associated with changes in NREM sleep (Scullin, Trotti, Wilson, Greer, & Bliwise, 2012). The ToH task depends heavily upon motor skills to be executed quickly and motor skill improvement on many such tasks is dependent upon NREM sleep (Morin et al., 2008). One study found that increased speed in a sequential finger-tapping task correlated with N2 sleep, especially in the last quarter of the night (Walker et al., 2002). In our study, the fact that ToH_{time} was correlated with N2 sleep to a greater extent than was ToH_{moves} and the fact that this association occurred especially in the 2nd half of the night, are both consistent with the notion that participants' improved ToH performance was due to a NREM sleep-mediated increase in motor speed. In related research, the ToH has been found to contain a substantial declarative memory component in that solution strategies may be explicitly remembered over time (Winter, Broman, Rose, & Reber, 2001). Declarative memory is widely known to be dependent upon NREM more than upon REM sleep (Walker & Stickgold, 2010) and so may be a factor in the ToH results. NREM sleep has also been found to mediate memory for various other executive processing tasks, including tasks on which participants expect to receive a subsequent retest (Wilhelm et al., 2011). Participants in the present study were not specifically told they would be retested but it is possible that they developed such expectations; experimenters in this study were not blind to the occurrence of retests.

Tasks that draw upon very different memory systems may block one another altogether. Sleep-related improvements on a procedural memory task are blocked if the task is followed closely by a declarative memory task, and vice versa (Brown & Robertson, 2007). In the present study, the CBT and ToH tasks may have been different enough in their memory demands that they interfered with each other in this manner. For example, the CBT task may have blocked consolidation of the prior ToH task. However, in such a case one would expect performance on the two tasks to be negatively correlated; yet correlations between the 2 tasks were negligible.

(3) *Participants differing in task aptitude*: A third explanation for why the ToH task alone was not associated with REM sleep as expected is that our participants may have been more verbally proficient than those in other studies. ToH performance is better in bilingual participants who have strong language control than it is in bilinguals who have weak language control; verbal intelligence is also higher in the former group (Festman, Rodriguez-Fornells, & Munte, 2010). Verbal memory performance has also been found to be dependent upon NREM sleep (Molle, Bergmann, Marshall, & Born, 2011). Since our participants were primarily University students living in the Montreal area, many may have had strong bilingual language skills and their improvement on the ToH task may therefore have depended proportionally more upon NREM sleep. Post-hoc analyses suggested the presence of a trait difference at play in the ToH group differences; this trait may have been related to verbal proficiency.

In a similar vein, our participants may have already possessed an elevated proficiency in solving puzzles similar to the ToH which, as indicated earlier, also includes a salient procedural component. In previous studies, participants judged to have high levels of pre-existing skill in a procedural task (rotor pursuit) showed a clear association between improvement in learning and N2 sleep whereas participants judged to have low levels of skill on the task showed an association with REM sleep (Peters, Smith, & Smith, 2007). This participant difference may extend to proficiency in simple motor performance as discussed earlier.

In sum, the present findings are not unique in demonstrating a mismatch between a given task and its expected sleep stage. As indicated in the Introduction, declarative memory tasks can at times be associated with REM (rather than NREM) sleep (Tilley & Empson, 1978) and non-declarative memory tasks can be associated with NREM (rather than REM) sleep (Doyon et al., 2009; Morin et al., 2008; Smith & MacNeill, 1994; Walker et al., 2002). To explain their own finding that REM sleep theta activity was associated with paired associate learning—a type of learning typically associated with NREM sleep (Fogel, Nader, Cote, and Smith, 2007)—Fogel, Smith, and Cote (2007) suggested that different types of learning may require consolidation mechanisms that act in dissociable brain regions at different times of the night. The present findings support such speculation to the extent that changes in spindle amplitude as well as increases in REM sleep were associated with overnight improvements on the CBT task. Additional analyses of REM and NREM microstructural events such as theta activity or slow oscillations could further test this possibility.

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The present findings suggest caution in interpreting results from experiments in which several types of memory are assessed simultaneously (e.g., item vs. context memory (van der Helm et al., 2011)). If such a study reports a sleep-dependent improvement on one task but not on another, the second task may yet prove to be sleep-sensitive in other circumstances, e.g., if administered alone. To the extent that two (or more) tasks administered in close temporal proximity compete for the same sleep-related resources, one may emerge as sleep-dependent in preference to another. Such a shift might be expected if a function of sleep is to protect memory from interference by competing tasks (Ellenbogen, Hulbert, Stickgold, Dinges, & Thompson-Schill, 2006). Similarly, a habitually REM sleep-dependent task could be induced to change its dependence to NREM sleep under some circumstances, as seems to have occurred in the present study.

4.1. Awakenings for dream reporting

Dream mentation sampling in the present study revealed a confound between the amount of REM sleep in the sleep period and the total number of awakenings effectuated for recall of REM, but not N2, dreaming. Both REM minutes and REM #awakenings were strongly associated with improvement on the CBT task, although covariate analyses revealed that the main effect for REM minutes persisted as a trend ($p = .068$), and especially for the 2nd half of the night ($p = .075$), when the REM #awakenings measure was covaried. Conversely, the REM #awakenings difference did not persist when REM minutes was covaried ($p = .148$). This, and the fact that REM #awakenings and REM minutes were only moderately correlated ($r = .491$), suggests that both variables may have an independent influence on CBT improvement. This possibility suggests caution in designing studies that use REM sleep awakenings to assess dream-related memory consolidation effects.

This is especially the case since the effect for #awakenings was not contingent upon whether dream mentation was recalled during those awakenings. The current recall levels (REM: 83.3%; N2: 66.8%) are only slightly higher than average levels estimated across 50 studies (REM: 79.5%; NREM: 42.8%) (Nielsen, 2011) but may have placed a ceiling on the sensitivity of this measure for REM sleep awakenings in particular. Nonetheless, the results suggest that awakenings from REM sleep themselves and not necessarily the recall of dream mentation following these awakenings may be implicated in improved learning. This is broadly consistent with results from animal studies showing that sequences of REM and NREM sleep that include transitions to wakefulness are associated with fast learning (Piscopo et al., 2001). And because participants usually return into N2 sleep after they have been awakened from REM sleep for dream-reporting, it is also possible that the timing of these post-awakening sleep transitions is implicated in CBT improvements—despite the demonstrated lack of association between CBT improvement and N2 sleep time per se.

Many have speculated that dreaming plays a role in offline memory consolidation (most recently Smith, 2010; Wamsley & Stickgold, 2011). This role is often sought by identifying experiential replays of task-related memories in dream content. However, such attempts have had limited success (Smith & Hanke, 2004). With the exception of sleep onset NREM imagery (Wamsley, Perry, Djonlagic, Reaven, & Stickgold, 2010), task-related dream content is most typically indirect, metaphoric or fragmentary in nature. It remains to be seen if non-specific dream content changes involving no apparent relation to task imagery are implicated in overnight learning, for example, a non-specific increase in dream emotionality or bizarreness. Such studies will continue to be confronted with the challenge of disentangling the confounding of REM sleep physiology, experimental awakenings for dream recall, and REM sleep dreaming measures.

4.2. Limitations of the study

A potential confound of the present findings, that the post hoc division of groups into improved vs. not improved participants reflects a trait factor influencing both sleep structure and task improvement, was supported to some extent by post hoc analyses of the ToH task results. Controlling for initial levels of performance on the most sleep-sensitive measure (ToH_{time}) reduced the sleep-dependent main effect on N2 sleep to a trend and eliminated correlations between %N2 sleep and cross-night improvement. These findings are consistent with others reporting relationships between EEG characteristics of N2 sleep—including sleep spindles—and trait characteristics such as performance IQ (Fogel, Nader, et al., 2007; Geiger et al., 2012). The present findings do not necessarily mean that N2 sleep characteristics are unrelated to task improvement in this cohort; we were simply unable to demonstrate clearly independent contributions for trait and sleep stage factors. Further assessment of N2 sleep architectural variables—sleep spindles in particular—may yet reveal independent trait and learning factors (Fogel & Smith, 2011). The present results should also be interpreted cautiously insofar as performance measures were associated with different, albeit interrelated, sleep measures. The fact that TOH improvement was associated with both increased N2 sleep and decreased N3 sleep may mean that the N3 decrease, too, was a necessary component of the improvement rather than an inevitable consequence of the N2 increase. In a similar fashion, the negative correlations between CBT improvement and spindle amplitude might reflect an interactive relationship between inhibited spindle generation and increased REM sleep time that is important for consolidation of this task.

Author contributorship

Nielsen: study design, data analysis/interpretation, manuscript writing; Carr, Dumel, Godin, Solomonova, Lara-Carrasco, Blanchette-Carrière: data collection/entry; O'Reilly, Paquette: data analysis/interpretation, manuscript writing.

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